Section 6
Reference Areas

6.1 Introduction

A total of five areas were selected to represent reference conditions within the Calcasieu Basin. These areas included Choupique Bayou, Grand Bayou, Bayou Bois Connine, Willow Bayou, and Johnsons Bayou (Figure 2-7). Within the five areas, 18 reference sites were selected using a stratified random sampling design, whereby each of the five reference sub-areas (strata) are sampled individually by simple random sampling. The reference areas were selected for minimal sediment contamination while having similar sediment characteristic as the Calcasieu Estuary study area as described in Section 2. Examination of these reference area sediments will enable the interpretation of the significance of elevated concentrations that were observed in the respective sediments from the study area.

6.2 Comparing Reference Sites Among the Reference Areas

Because the reference sites were stratified into five different reference sub-areas, of primary concern was comparing observations from the reference sites to determine whether all of the reference sites could be considered to come from the same population, i.e., to determine whether the distributions of chemical concentrations in each of the five reference sub-areas were the same. The nonparametric one-way Analysis of Variance (ANOVA) is a commonly used statistical method to compare two or more groups, in this case reference sites, collectively. The nonparametric ANOVA is also called the Kruskal-Wallis test. For comparative purposes, only chemical constituents in the sediments were examined, because surface water tends to be fairly variable both spatially and temporally.

6.3 Kruskal-Wallis Test

Comparisons between the five reference areas were conducted using the Kruskal-Wallis test on selected constituents (See Appendix C). The Kruskal-Wallis test evaluates whether three or more sample distributions are derived from the same population, or whether there is a statistically significant difference between any two of the three or more sample distributions. The null hypothesis (H_0) and the alternative hypothesis (H_A) can be formulated as follows:

- H_0: All five reference area sample distributions are from the same population against the alternative.

- H_A: At least one of the five reference area sample distributions are from a different population that at least one other reference area sample distribution at a significance level α =0.05.

The Kruskal-Wallis test is described in detail in Gilbert (1987) and EPA (1992). The test was conducted on reference area sediment samples using the computer software Systat® Version 10.

The Kruskal-Wallis test is an extension of the nonparametric Wilcoxon ranked sum test for comparing more than two groups simultaneously, just as the parametric one-way ANOVA is an extension of the parametric t-test. As with the Wilcoxon ranked sum test, the Kruskal-Wallis test does not require the assumption that the data are derived from a normal (or any other specific) population distribution. The only assumption is that the distributions have the same shape, although they need not be symmetric. The Kruskal-Wallis test was selected (over the parametric one-way ANOVA equivalent) because the population distributions were unknown and the sample sizes of the data were generally insufficient to distinguish the shape of the population distribution. In addition, the Kruskal-Wallis test is capable of handling the moderate number of nondetect values present in the sample data. Both parametric and nonparametric ANOVA are based on the idea that if the amount of variability between the sample distributions is large relative to the amount of variability within each distribution, thus providing an indication that the distributions are not derived from the same population.

6.4 The Selection of Chemical Constituents

To ensure consistency, the sediment sample data used for the Kruskal-Wallis test were obtained using the same protocols employed in the PCA and the Wilcoxon ranked sum tests. The data groups consisted of the reference sample sites distributed among the five reference areas as displayed in Exhibit 6-1.

Exhibit 6-1 Distribution of Reference Sites Among the Reference Area.

Reference Area	Reference Sites			Energy Area
Bayou Bois Connine	SNST006	SNST035		Bayou
Bayou Choupique	SNSTO11 SNST014	SNST012 SNST015	SNST013	Bayou
Grand Bayou	SNST008	SNST009	SNST038	Bayou
Johnsons Bayou	ECOSN001 SNST003	ECOSN002 SNST004	ECOSN003 SNST005	Bayou
Willow Bayou	SNST001	SNST002		Bayou

The chemical constituents selected for the Kruskal-Wallis testing were determined from the PCA results and were the same as those selected for the Wilcoxon ranked sum testing. These chemical constituents are depicted in Exhibit 6-2. All but the last of the constituents were selected directly from the PCA results. The last chemical constituent, 2,3,7,8-TCDD toxicity equivalent, was selected based on the prevalence of dioxin/furan isomers in the PCA results. The dioxin/furan isomers were converted to 2,3,7,8-TCDD toxicity equivalents (TEQ) as described in Section 4, then summed to obtain the total 2,3,7,8-TCDD toxicity equivalents.

Exhibit 6-2 Selected chemical constituents.

Chemical Constituent	Frequency of Detect	Minimum Detected Value	Maximum Detected Value	Mean	Standard Deviation	Coefficient of Variation
2,3,7,8-TCDD Equivalent (pg/g)	5/5	3.39	7.08	4.3	2.1	0.5
Aroclor 1254 (μg/Kg)	3/18	100	1000	---	---	---
Arsenic (mg/Kg)	3/3	4.8	9.5	8	3	0.4
Barium (mg/Kg)	3/3	28.7	40.1	35	7	0.2
bis(2-Ethylhexyl)phthalate (μg/Kg)	5/18	55	110	71	16	0.2
Chromium (mg/Kg)	3/3	7.9	10.5	9	1	0.2
Copper (mg/Kg)	18/18	4.9	18.1	13	3	0.3
Lead (mg/Kg)	18/18	8.6	29.1	19	6	0.3
Mercury (mg/Kg)	15/18	0.020	0.093	0.06	0.02	0.37
Nickel (mg/Kg)	18/18	8.5	21.5	16	5	0.3
Zinc (mg/Kg)	18/18	36	86	60	15	0.3
Pyrene (μg/Kg)	3/6	25	80	42	29	0.7

6.5 Kruskal-Wallis Results

The Kruskal-Wallis tests were conducted to compare each chemical constituent in each reference area to that chemical constituent in all other reference areas. Since there were six constituents, a total of six tests were conducted. The results are provided in Exhibit 6-3.

Exhibit 6-3 provides the probability (or p-value) results of the Kruskal-Wallis comparison test. The p-values must be compared with a critical value (alpha, α) in order to determine whether there is a statistical discernable difference between the five reference area groups tested. For the purpose of interpreting the p-values, three levels of significance were established:

- p-value > α accept H_0: All five reference areas are derived from the same population.
- p-value < α reject H_0: At least one of the five reference areas may be derived from a different population than at least one other reference area.
- adjusted p-value < α reject H_0: At least one of the five reference areas is derived from a different population than at least one other reference area.

For the first two levels of significance (all five reference areas are derived from the same population and at least one of the five reference areas may be derived from a different population than at least one other reference area), the p-values were compared directly with α = 0.05. For the third level of significance (at least one of the five reference areas is derived from a different population than at least one other reference area), an adjusted p-value was used based on the total number of comparison tests conducted. The adjusted p-value was calculated using the Bonferroni adjustment method:

- adjusted p-value = n(p-value) where n is the number of comparison tests conducted.

In Exhibit 6-3, adjusted p-values that were less than alpha (α) are shown in parentheses; these are considered cases where the Kruskal-Wallis test definitely indicates that at least one of the five reference areas is derived from a different population than at least one other reference area.

Exhibit 6-3 Kruskal-Wallis Test Results – Comparison Among Reference Areas.

Chemical Constituents	**p-Value** [1]
Arochlor 1254 (μg/Kg)	0.074
Bis(2-Ethylhexyl)phthalate (μg/Kg)	0.113
Pyrene (μg/Kg)	- - -[2]
2,3,7,8-TCDD TEQ (pg/g)	0.202
Lead (mg/Kg)	**0.013**
Mercury (mg/Kg)	**0.042**
Nickel (mg/Kg)	**0.009** (0.045)
Arsenic (mg/Kg)	- - -[2]
Barium (mg/Kg)	- - -[2]
Chromium (mg/Kg)	- - -[2]
Copper (mg/Kg)	0.298
Zinc (mg/Kg)	**0.007** (0.035)

[1] If p-value < critical value (α = 0.05) then there is a statistically significant difference between the reference areas. Bold = significant difference. Values in parentheses are Bonferroni adjusted p-values (shown only if < 0.05).
[2] Insufficient data for analysis.

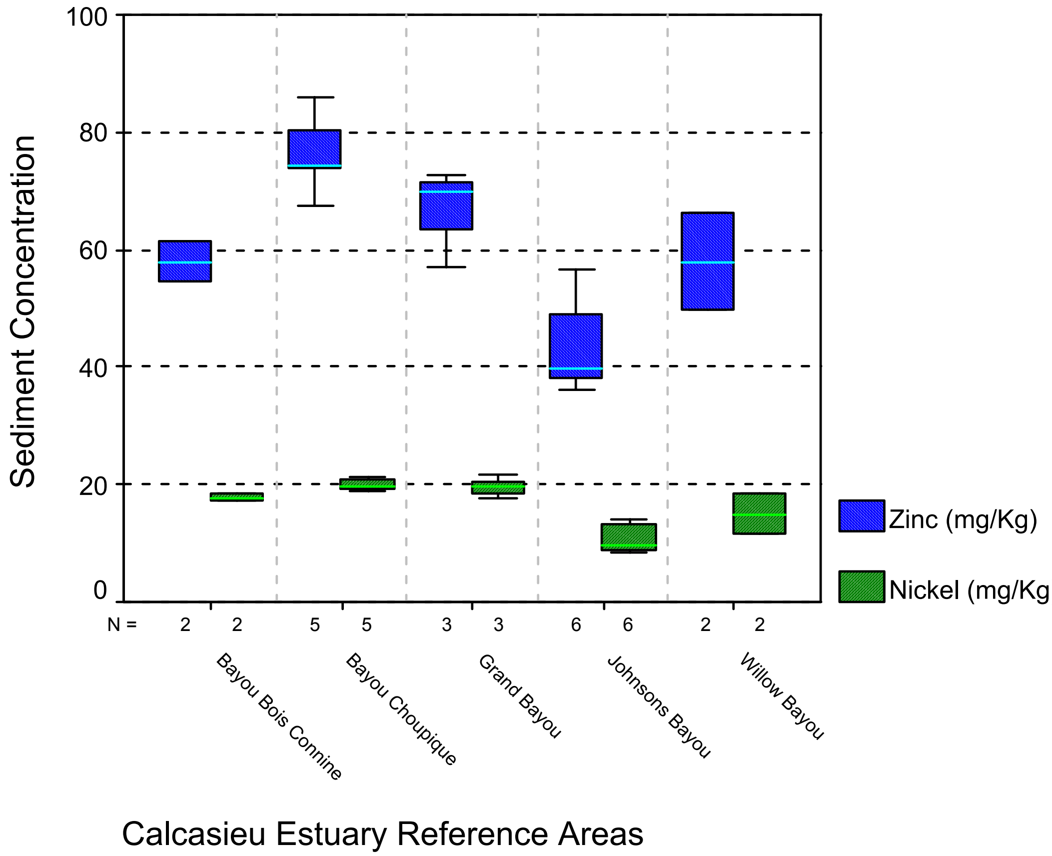
6.6 Conclusions

As illustrated in Exhibit 6-3, only nickel and zinc produced adjusted p-values less than 0.05, indicating at least one reference area is derived from a different population than at least one other reference area. Although lead and mercury yielded p-values of 0.013 and 0.042 respectfully, the adjusted p-values indicated that there was no statistically significant difference between the reference areas for the two chemical constituents.

Whereas shown in Exhibit 6-4, the low p-value attributed to zinc is due to a significantly higher zinc distribution in Choupique Bayou compared with Johnsons Bayou. This could be indicative of low-level zinc contamination in Choupique Bayou (specifically around reference site SNST011); however, the concentrations are low and could represent elevated natural background zinc levels in Choupique Bayou relative to Johnsons Bayou. Likewise, the adjusted p-value attributed to nickel is probably due to a significantly higher nickel distribution in all of the reference areas when compared to Johnsons Bayou. Since the concentrations for nickel were low, less than

22 mg/Kg for all locations and with a range of about 12 to 22 mg/Kg for four reference areas, this could also represent natural background levels in all of the reference areas relative to Johnsons Bayou. Additionally, Johnsons Bayou tended to exhibit lower constituent concentrations compared to other areas.

Exhibit 6-4 Comparison Among Reference Areas for Nickel and Zinc.



Furthermore, as pointed out in Exhibit 6-4, the other three reference areas had consistent zinc concentrations ranging between about 50 and 72 mg/Kg. Given that this range likely represents variation in natural background, and since no significant differences exist for other chemical constituents, the results of the Kruskal-Wallis test indicate that the five reference areas are similar and can be combined for the purpose of comparison with the other Calcasieu Estuary study areas/energy areas.